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2.	Patent application number *(The Patent Office will fill in this part)*	**0003282.1**
3.	Full name, address and postcode of the or of each applicant *(underline all surnames)*	TEPNEL MEDICAL LIMITED UNIT 8, ST GEORGE'S COURT HANOVER BUSINESS PARK ALTRINCHAM WA14 5UA
	Patents ADP number *(if you know it)*	4449542002
	If the applicant is a corporate body, give the country/state of its incorporation	
4.	Title of the invention	ISOLATION OF BIOMOLECULES
5.	Name of your agent *(if you have one)*	Marks & Clerk
	"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*	Sussex House 83-85 Mosley Street Manchester M2 3LG
	Patents ADP number *(if you know it)*	18004
6.	If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number	Country / Priority application number *(if you know it)* / Date of filing *(day/month/year)*
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8.	Is a statement of Inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if: a) any applicant named in part 3 is not an inventor, or b) there is an inventor who is not named as an applicant, or c) any named applicant is a corporate body. See note (d))*	YES

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	Specification	Appendix
Continuation sheets of this form	-	-
Description	14	14
Claim(s)	5	5
Abstract	-	-
Drawing(s)	3	1

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Priority documents	-
Translations of priority documents	-
Statement of Inventorship and right to grant of a patent *(Patents Form 7/77)*	-
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ISOLATION OF BIOMOLECULES

The present invention has a number of separate aspects all of which relate, in general terms, to improvements in obtaining a biomolecule (e.g. a protein or nucleic acid) from a cell sample.

Numerous methods are known for obtaining biomolecules, for example nucleic acids and proteins, from biological material such as viruses, bacterial and eukaryotic cells, cell aggregates and tissue or body fluids. Typically the biomolecule to be obtained is a soluble molecule and is "released" from the biological material by a lysis procedure (e.g. alkaline lysis) resulting in a suspension comprised of a solution of the target biomolecule also containing soluble proteins, carbohydrates, fats, amino acids and other metabolites from the disrupted cells. Thus, a process for obtaining a biomolecule from a cell sample will generally comprise the steps of

(i) cell lysis by addition to the cell sample of lysis solution possibly followed by addition of a neutralising solution;

(ii) optional, but generally desirable, separation of insoluble material from the lysed sample to provide a solution from which the biomolecule is "extracted";

(iii) immobilisation of the biomolecule onto a solid support usually in the presence of a chaotropic salt;

(iv) washing of the support; and

(v) elution of the immobilised biomolecule from the support.

The cell sample to which (i) refers may for example be obtained by centrifugation of a culture of cells, separating the cells from the supernatant, and re-suspending the cells in re-suspension buffer.

First Aspect

The first aspect of the invention relates to improvements in a method of operating automated apparatus for obtaining a biomolecule from a cell sample. The apparatus with which this aspect of the invention is concerned is one in which various, but not necessarily all, of the steps in obtaining the biomolecule are effected in disposable columns which are removably mounted on the apparatus.

The apparatus employed in the method of this aspect of the invention will comprise at least the features of

(a) a head arrangement having a plurality of individual column supporting heads on which the upper ends of disposable columns may be removably mounted and which have fluid flow passageways for transfer of fluids into and out of the upper ends of the columns;

(b) means for moving a supply of disposable columns so that upper ends of columns to be mounted on the supporting heads are presented below said heads;

(c) means for moving the head arrangement relatively downwardly towards the tops of the columns whereby the upper ends thereof become removably mounted on said head, and for moving the head arrangement relatively upwardly with columns mounted thereon in readiness for subsequent stages of the process.

(d) reservoirs for reagents/wash solutions as appropriate;

(e) means (e.g. for providing pressure variation in the columns) for causing liquid samples, liquid extracts or mixtures of such liquids with reagents thereof, provided at the lower tips of the columns (e.g. in wells of a microlite plate, BioBlock or similar) to be drawn upwardly into the columns and discharged from the lower tips thereof for processing steps as required to obtain the desired biomolecule; and

(f) means for removing the columns from the heads for disposal.

Such an apparatus is referred to herein as an "apparatus of the kind defined".

An example of a technique using disposable columns, and an apparatus of the kind defined in our earlier UK patent application No. 9917299.1 whereof a copy of the specification is attached to this specification as an Appendix for purposes of full disclosure.

Thus, the apparatus of the kind defined may be operated in accordance with the sequence of processing steps as described more fully in the Appendix. As a variation of the sequence defined in that application (in which the disposable column is mounted on the column supporting head with removable filter attached to the column) the disposable column may initially be mounted on the supporting head and the filter subsequently mounted in the lower end of the column using the means (b) and (c) operated as appropriate.

In accordance with the first aspect of the present invention there is provided a method of operating an apparatus of the kind defined using the steps of lysing the cell sample and obtaining the biomolecule from the lysate wherein reagents for lysis are ejected downwardly through the supporting heads into the cell sample to be lysed (either prior or subsequent to mounting columns on the heads).

The lysis reagents will comprise a lysis solution (e.g. incorporating dodecyl sulphate) and generally also a separate neutralising solution as well known in the art, which will be ejected separately of the lysis solution.

We have found that downward ejection of the lysis reagents into the cell sample avoids the need for any further mixing which considerably simplifies the overall extraction procedure and construction of the apparatus. It should however be noted that to achieve this advantage the lysis reagents should be discharged directly

into the cell sample without initially impinging on the wall of the vessel in which the sample is contained.

In a particularly preferred embodiment of this aspect of the invention, the cell samples to be lysed are contained within the individual wells of a microtitre well tray or similar (e.g. BioBlock) and the lysis reagents are ejected into the individual wells. Either single or multiple aliquots of lysis solution and neutralising solution may be discharged into each well. In a particularly convenient form of the apparatus, there is a single row of n (e.g. 12) column supporting heads and the cell samples to be processed are held in wells of a microtitre plate or similar in which the wells are arranged as m rows each of n wells (i.e. an m x n array, e.g. 8 x 12). In such an apparatus, the samples in the n wells of any one of the m rows are simultaneously treated with lysis reagents (by downward ejection from the n supporting heads). It will generally be preferred that the lysis reagents are discharged into all wells of the (m x n) array prior to the disposable columns being mounted on the heads for subsequent steps of the extraction procedure. Thus, lysis agents may initially be added to the n wells of the first of the m rows of the microtitre plate or similar, subsequently the plate is moved relative to the heads so that the second of the m rows is below the heads for introduction of lysis reagents into these wells and so on until the m rows of wells have been treated with the lysis solution. If desired, the above procedure may then be repeated for the introduction of neutralising solution (via the heads) into the wells. The disposable columns may then be mounted on the supporting heads and all steps of the extraction procedure effected for the samples in the first row of wells prior to replacement of the columns, processing of the second row and so on until all wells of the (m x n) array have been processed.

In a modification of the procedure, columns may be mounted on the heads prior to dispense of the lysis and/or neutralisation reagents.

The time for which the lysis reagents are dispensed into each of the wells may for example be 0.1 to 1 seconds although this will depend in volume to be dispensed (typically 150μl of lysis solution and 300μl neutralisation solution) and pump speed.

The discharge outlet of the column supporting head may be about 40mm to 100mm (preferably about 70mm) above the surface of the sample into which the lysis reagents are to be discharged. Typically also the discharge outlet of each supporting head will have a diameter of 0.1 to 1 mm (preferably about 0.5mm).

It should however be noted that all of the values set out in the previous paragraph are exemplary and other values, which may readily be evaluated, may be required depending on the actual construction of the apparatus.

Second Aspect

The second aspect of the invention relates to improvements in the filter unit for a biomolecule purification assembly of the type which comprises (i) an elongate vessel in the form of a column having first and second open ends with a filtrate inlet being provided at the first end of the column, and (ii) the filter unit itself being comprised of a sleeve which is removably located over the first end of the vessel and which houses a filter. An example of such a biomolecule purification assembly is disclosed in our aforementioned copending application (see Appendix).

According to a second aspect of the present invention there is provided a filter unit for a biomolecule purification assembly, said filter unit comprising

(a) an elongate sleeve having one end (the "suspension inlet end") through which a suspension to be filtered enters the sleeve and an opposite end for location over a column of a biomolecule purification assembly, said sleeve having a first body portion and a second body portion which internally tapers from the first body portion to said suspension inlet end, and

(b) a filter having a head portion locating in, and occupying the cross-section of, the first body portion, and a frustoconical or conical body portion extending into said second body portion of the sleeve and tapering therein at a larger angle than the internal taper of the sleeve.

The construction of filter unit as defined in the previous paragraph has a number of advantages as detailed below.

Firstly, there is clearance between the outer surface of the conical or frustoconical portion of the filter and the inner wall of the tapering portion of the sleeve. This ensures that there is a relatively large area of the filter exposed to the suspension to be filtered so that the filter unit has as high a capacity for filtration as possible given the overall dimensions of the filtration unit.

Secondly, the "dead space" within the filter unit (i.e. the space between the outer surface of the conical or frustoconical portion of the filter and the tapering section of the sleeve) may be relatively low whilst still allowing the advantage of the previous paragraph. The minimum dead space ensures that there is a minimum liquid remaining (in the dead space) when all of the suspension to be filtered has been drawn up into the filter unit and air is about to enter the column.

Thirdly, the elongate nature of the filter unit ensures that it's lowermost, suspension inlet end may be juxtaposed to the base of a microtitre well or the like.

Overall, therefore, the filter unit allows maximum amount of suspension to be filtered, with high filtration efficiency, before air enters the unit.

Preferably the first body portion of the sleeve is of circular internal cross-section and the head of the filter is also cylindrical so as to be a close fit therein.

It will be appreciated that the filter unit of this second aspect of the invention may be employed as the filter unit in a biomolecule purification as otherwise described and defined (e.g. in claim 23) in the accompanying Appendix.

Third Aspect

At the end of the procedure (e.g. as described in the Appendix) for obtaining the desired biomolecule, the "product" in each well of the microtitre plate (or similar) is a solution of the extracted molecule in liquid(s) introduced during the process ("process liquid(s)"). If however one, other or both of the lysis and neutralisation agents have not been added to the cell sample being extracted, the "product" in the wells will simply comprise the "process liquids" and not the desired biomolecule. In this latter case, the "product" will be usually indistinguishable from that intended (i.e. a liquid containing the desired biomolecule). Thus it will not be immediately apparent that the extraction process has failed for want of addition of lysis and/or neutralising solution.

According to a third aspect of the present invention there is provided a method of obtaining a sample of a biomolecule from a cell suspension by a process which involves

(a) cell lysis by addition to the cell suspension of a lysis solution and neutralisation solution;

(b) filtration of the lysed suspension with a biomolecule purification assembly incorporating a removable filter;

(c) removing the filter; and

(d) processing the filtrate to extract the desired biomolecule

wherein at least one of the lysis solution, neutralising solution and cell suspension incorporates an agent which produces a visually perceptible colour change only if the lysis and neutralising solutions are added to the suspension.

Steps (a) to (d) of this aspect of the invention may be effected as described in the Appendix.

Thus it is proposed in accordance with the third aspect of the invention that a visually perceptible colour change is produced only if both lysis solution and neutralising solution are added to the cell suspension.

Assuming that the colour change has occurred then during the filtration step there will be some coloration of the filter unit and/or the precipitate thereon. As described in the Appendix, it is preferred that the filter units are discarded into the microtitre wells (or similar) in which the samples of cell suspension were initially contained. It is therefore a simple matter visually to inspect the discarded filter units for evidence of colour and therefore confirmation that both lysis solution and neutralisation have been added.

The colour which is generated is preferably a soluble colour so that the filtrate actually colours the filter. As such, the colour may be observed on the inside surface of the filter by inspection of the microtitre plate from above. It is however possible for the colour generating agent(s) to produce a coloured precipitate which collects on the outer surface of the filter although this may be more difficult to observe from above and difficult to observe from the side of the microtitre plate, particularly those filter units which are in wells away from the edges of the plate.

In one embodiment of this aspect of the invention, the lysis solution will incorporate dodecyl sulphate and the neutralising solution incorporates a transition metal ion (eg Cu^{2+}, Fe^{2+}) capable of forming a coloured precipitate with dodecyl sulphate, the coloration of the precipitate only being produced if both lysis and neutralisation solutions are added.

Alternatively the lysis solution may incorporate a pH indicator which changes colour if the neutralising solution is added.

A further possibility is that the cell suspension to be lysed incorporates a pH indicator (which may have been provided the re-suspension buffer) which changes to a given colour if both lysis and neutralisation solution are added.

Fourth Aspect

The fourth aspect of the invention relates to the disposables for use in an apparatus of the kind defined. These disposables include the disposable columns and the disposable filter units which locate on the columns to form a biomolecule purification assembly (see also definition for the second aspect of the invention).

As explained for the first aspect of the invention, the columns (with or without filter unit previously located thereon) are mounted on the apparatus by virtue of the head arrangement moving relatively downwardly towards the tops of the columns whereby the upper ends thereof become removably mounted on the head. If the filter units are to be mounted subsequently onto the lower ends of the columns then the filter units are positioned below the columns and the head arrangement in moved relatively downwardly so as to cause the filter units to be push-fitted into the lower ends of the columns.

According to the fourth aspect of the present invention there is provided a supply unit of disposables for loading onto an apparatus of the kind defined in which said disposables are

(i) open-ended columns,
(ii) filter units for mounting on the open-ended columns, or
(iii) biomolecule purification assembles formed from the column (i) having the filter units (ii) mounted thereon,

said supply unit comprising a n x m array of the disposables supported in respective apertures of a top member of a base assembly having internal reinforcing elements adapted to resist compressive forces applied in the axial direction of said disposables.

The reinforcement in the base unit ensures that the supply unit is able to withstand the downward pressure exerted by the head arrangement (which for a head arrangement incorporating a line of 12 column supporting heads this downward force may be 15kg across the 12 heads) when mounting the disposables on the apparatus without collapse of the base unit. Additionally the base unit is able to withstand the cumulative effects of the force involved in multiple "pick-ups" of the disposables from the base unit. It is important that the base unit withstands these forces since the movement of the head arrangement relatively towards the upper ends of the disposables will be a movement pre-programmed on the basis of a required, accurate positioning of these upper ends. Collapse of the base unit would mean that the upper ends of the disposables were not in the correct position for loading onto the apparatus.

The n x m array may for example be a 12 x 8 array or an 8 x 8 array.

The reinforced elements may be in the form of a grid.

The reinforcing elements are preferably lamellae (e.g. strips) and have a widthwise edge level with the bottom of the base assembly.

The base assembly may, for example, be generally square or rectangular in plan view and thus be comprised of four side elements and the aforementioned top member.

The reinforcing elements may comprise a first set of such reinforcing elements extending (within the base assembly) between two opposed side elements and a second set of such reinforcing elements extending transversely (e.g. perpendicularly) to the first set between the other two opposed sides.

For ease of manufacture, the base assembly may be formed of

(i) a lower deck which has four side elements and which incorporates the reinforcing elements; and

(ii) an upper deck which has four side elements so as to be mountable on the lower deck and which incorporates the aforementioned apertured top member.

It will be appreciated that the various aspects of the invention may be used in any combination as appropriate.

Certain aspects of the invention will now be further described, by way of example only, with reference to the accompanying drawings, in which:

Fig 1 is an exploded view, to an enlarged scale, a biomolecule purification assembly incorporating a filter unit in accordance with the second aspect of the invention;

Fig 2 illustrates to a still further enlarged scale, the filter incorporated in the filter unit illustrated in Fig 1;

Fig 3 illustrates a modification of the filter unit shown in Fig 2;

Figs 4a and 4b illustrate an embodiment of supply unit in accordance with the fourth aspect of the invention; and

Figs 5a and 5b illustrate a further embodiment of supply unit in accordance with the fourth aspect of the invention.

Referring firstly to Fig 1, there is illustrated a biomolecule purification assembly 101 for use in obtaining a purified sample of a biomolecule of interest from a suspension (e.g. as obtained by an alkaline lysis procedure) comprising a solution of the biomolecule containing insoluble biological debris. The procedure may, for example, be as described in the Appendix. The illustrated assembly 101 comprises a vertically disposed. open-ended column 102 and a filter unit 103.

The column 102 is referenced for convenience as being comprised of body sections 104, 105, 106 and 107. Body section 104 defines an upper cylindrical bore 104a which at its lower end is connected to a downwardly tapering section 105a leading into a lower bore 106a (in body section 106) which is of reduced diameter as compared to bore 104a. At its lower end bore 106a leads into a tapering section 107a defined within the lower section 107 of the column 102.

The lower end of tapering section 107a defines a filtrate inlet for the column.

A shoulder 105b is defined around body section 105.

At the upper end of column 102 there is provided a formation 109 by means of which the column may be mounted on the supporting head of a sample processing apparatus of the type described more fully above.

The filter unit 103 is a two component part and comprises a sleeve 110 within which is housed a filter 111.

The sleeve 110 has a first body portion 112 within which is defined a cylindrical bore 113 having an upper end 113a by means of which the filter unit 103 is mounted on the lower end of column 102. The second body portion 114 has an internal bore 115 which internally tapers from the first body portion 112 to a suspension inlet 116 of the filter unit 103.

At the transition of the bore 113 into bore 116 is a shoulder 117.

The filter 111 (see also Fig 2) comprises a cylindrical head portion 118 and frustoconical body portion 119. At the transition of head portion 118 into body portion 119 is a shoulder 120.

The filter unit 111 locates in sleeve 103 such that the head portion 118 (of the filter) occupies the full cross section of the bore 113 (of sleeve 103), the shoulder 120

(of filter 111) seats on the shoulder 117 (within sleeve 112) and the frustoconical body portion 119 (of the filter) extends into the second body portion 114 (of the sleeve 103) so as to taper therein towards its suspension inlet end 116.

The angle at which the frustoconcical body portion 119 (of the filter 111) tapers is greater than the angle of taper of the bore 115 (of the second body portion 114 of the sleeve 103) so that there is clearance between the outer surface of the frustoconical body portion 119 and the inner, tapering surface of the second body portion 114 of the sleeve 103. This arrangement ensures that there is a relatively large area of the filter exposed to the suspension to be filtered so that the filter unit has a high a capacity for filtration as possible given the overall dimensions of the filtration unit.

Furthermore, the "dead space" within the filter unit 103 (i.e. the space between the outer surface of the frustoconical body portion 119 of the filter 111 and the inner surface of the tapering bored 105) is relatively low whilst still allowing the advantage of the previous paragraph. This "low volume" dead space ensures that there is minimum liquid remaining in the dead space when all of the suspension to be filtered has been drawn up into the filter unit and air is about to enter the column.

At the top of the filter 111 is a generally hemispherical depression 121 which generally serves to improve the flow through the filter. In the assembled biomolecule purification unit, the filtrate inlet for the column 102 is level with the top of the hemispherical depression 121. The depression 121 is however optional and is omitted in the filter illustrated in Fig 3 which is otherwise identical to that of Fig 2.

Referring now to Figs 4a and 4b, the former is an exploded perspective view of one embodiment of supply unit 200 in accordance with the fourth aspect of the invention and the latter is an assembled view.

As shown mostly clearly in Fig 4a, the supply unit 200 comprises a 12 x 8 array of disposable columns 201 (e.g. of the type depicted as 102 in Fig 1) supported on a base assembly 202 and enclosed by a lid 203.

Base assembly 202 is formed as upper and lower decks 204 and 205 respectively. Upper deck 204 is generally square in plan view and is comprised of side elements 206 and a top member 207 formed with a 12 x 8 matrix of apertures 208 within which the columns 201 locate. More particularly, the columns 201 have shoulders 201a (equivalent to shoulder 105b of column 101-see Fig 1) by means of which the columns are supported in the apertures.

Upper deck 204 seats on lower deck 205 which is comprised side elements 209a-d and reinforcing elements 210 and 211 formed as a grid. More particularly, there are

(i) a pair of the reinforcing elements 210 extending in parallel to each other between side elements 209a and 209c; and
(ii) a pair of the elements 211 extending in parallel to each other between side elements 209b and 209d and thus perpendicularly to the elements 210.

The supply unit shown in Figs 4a and 4b provides the advantages discussed more fully above in respect of the fourth aspect of the invention.

Reference is now made to Figs 5a and 5b which show a supply unit 300 generally similar to that illustrated in Figs 4a and 4b. There are however two differences. Firstly, the supply unit 300 comprises an array of filter units 301 (e.g. similar to the filter unit 103 shown in Fig 1) rather then columns. Secondly, the upper deck 304 is shallower than the upper deck 204 of the supply unit 200.

Claims

1. A method of operating an apparatus of the kind defined using the steps of lysing the cell sample and obtaining the biomolecule from the lysate wherein reagents for lysis are ejected downwardly through the supporting heads into the cell sample to be lysed (either prior or subsequent to mounting columns on the heads).

2. A method as claimed in claim 1 wherein the lysis reagents comprise a lysis solution and a separate neutralising solution.

3. A method as claimed in claim 1 or 2 wherein the cell samples to be lysed are contained within the individual wells of a microtitre well tray or the like and the lysis reagents are ejected into the individual wells.

4. A method as claimed in claim 3 wherein the apparatus comprises a single row of n column supporting heads and the cell samples to be processed are provided in an (m x n) array of microtitre wells wherein the samples in the n wells of any one of the m rows are simultaneously treated with lysis reagents by downward ejection from the n supporting heads.

5. A method as claimed in claim 4 wherein the lysis reagents are discharged into all wells of the (m x n) array prior to disposable columns being mounted on the heads for subsequent steps of the extraction procedure.

6. A method as claimed in any one of claims 1 to 5 wherein the time of dispense for the lysis reagent into a cell sample is 0.1 to 1 second.

7. A method as claimed in any one of claims 1 to 6 wherein the discharge outlet of the column supporting heads is 40mm to 100mm above the surface of the sample into which the lysis reagents are to be discharged.

8. A method as claimed in claim 7 wherein said discharge outlet is about 70mm above said surface of the sample.

9. A method as claimed in any one of claims 1 to 8 wherein the discharge outlet of each supporting head has a diameter of 0.1 to 1mm.

10. A method as claimed in claim 9 wherein said diameter is about 0.5mm.

11. A filter unit for a biomolecule purification assembly, said filter unit comprising

(a) an elongate sleeve having one end (the "suspension inlet end") through which a suspension to be filtered enters the sleeve and an opposite end for location over a column of a biomolecule purification assembly, said sleeve having a first body portion and a second body portion which internally tapers from the first body portion to said suspension inlet end, and

(b) a filter having a head portion locating in, and occupying the cross-section of, the first body portion, and a frustoconical or conical body portion extending into said second body portion of the sleeve and tapering therein at a larger angle than the internal taper of the sleeve.

12. A biomolecule purification assembly comprising

(i) an elongate vessel in the form of a column having first and second open ends with a filtrate inlet being provided at the first end of the column, and

(ii) the filter unit as defined in claim 11 removably located over the first end of the vessel.

13. An assembly as claimed in claim 12 wherein the filter unit has an upper depression and said filtrate inlet of the elongate vessel is located in the region of said depression.

14. An assembly as claimed in claim 12 or 13 wherein said elongate vessel is as defined in claim 23 of the Appendix.

15. A method of obtaining a sample of a biomolecule from a cell suspension by a process which involves

(a) cell lysis by addition to the cell suspension of a lysis solution and neutralisation solution;

(b) filtration of the lysed suspension with a biomolecule purification assembly incorporating a removable filter;

(c) removing the filter; and

(d) processing the filtrate to extract the desired biomolecule

wherein at least one of the lysis solution, neutralising solution and cell suspension incorporates an agent which produces a visually perceptible colour change only if the lysis and neutralising solutions are added to the suspension.

16. A method as claimed in claim 15 wherein the colour which is generated is a soluble colour.

17. A method as claimed in claim 15 or 16 wherein the lysis solution incorporates as pH indicator which changes colour if neutralising solution is added.

18. A method as claimed in claim 15 or 16 wherein the cell suspension to be lysed incorporates a pH indicator which changes to a given colour if both lysis and neutralisation solution are added.

19. A method as claimed in claim 15 wherein said agent(s) generates a coloured precipitate.

20. A method as claimed in claim 19 wherein the lysis solution incorporates dodecyl sulphate and the neutralising solution incorporates a transition metal in capable of forming a coloured precipitate with dodecyl sulphate.

21. A method as claimed in claim 20 wherein said transition metal in is selected from Cu^{2+} and Fe^{2+}.

22. A supply unit of disposables for loading onto an apparatus of the kind defined in which said disposables are

(i) open-ended columns,
(ii) filter units for mounting on the open-ended columns, or
(iii) biomolecule purification assembles formed from the column (i) having the filter units (ii) mounted thereon,

said supply unit comprising a n x m array of the disposables supported in a respective apertures of a top member of a base assembly having internal reinforcing elements adapted to resist compressive forces applied in the axial direction of said disposables.

23. A unit as claimed in claim 22 wherein the reinforcing elements are provided in the form of as grid.

24. A unit as claimed in claim 22 or 23 wherein the reinforcing elements are lamellae and have a widthwise edge level with the bottom of the base assembly.

25. A unit as claimed in any one of claims 22 to 24 wherein the base assembly is generally square or rectangular in plan view and wherein said reinforcing elements comprise a first set of such elements extending (within the base assembly) between two opposed side elements and a second set of such reinforcing elements extending transversely to the first set between the other two opposed side.

26. A unit as claimed in any one of claims 22 to 25 wherein the base assembly comprises

(i) a lower deck which has four side elements and which incorporates the reinforcing elements; and

(ii) an upper deck which has four side elements so as to be mountable on the lower deck and which incorporates the aforementioned apertured top member.

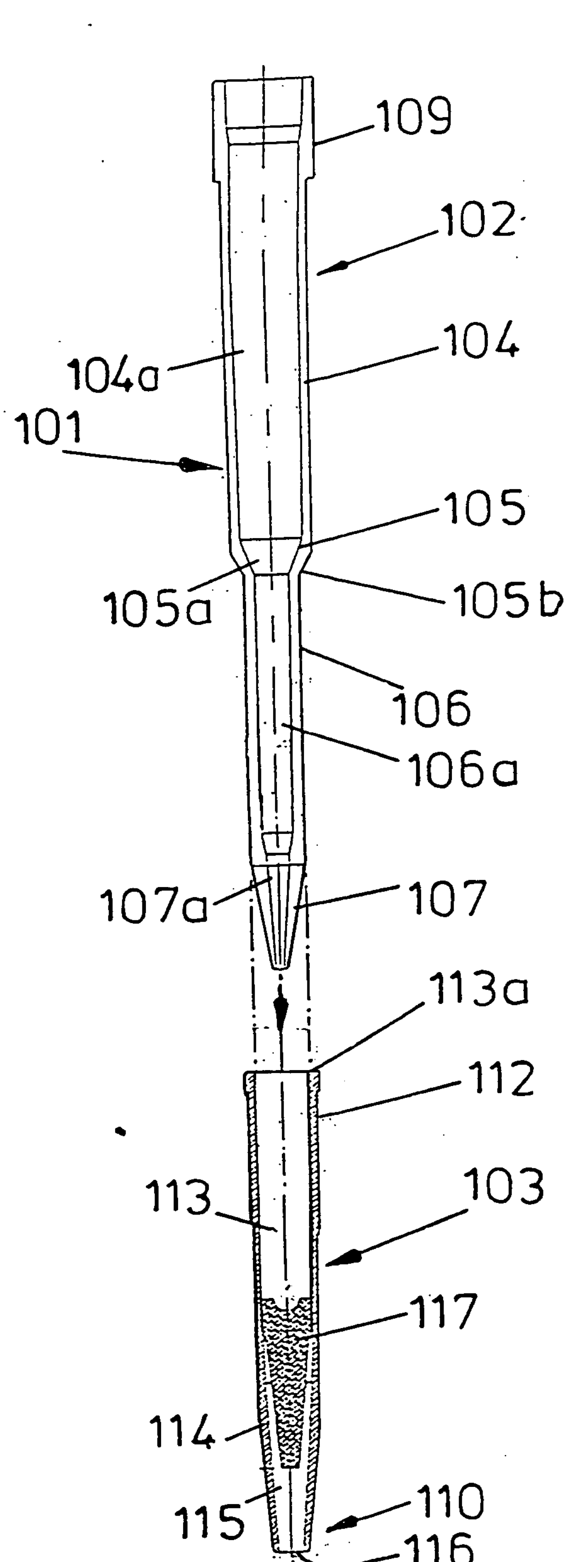

FIG. 1

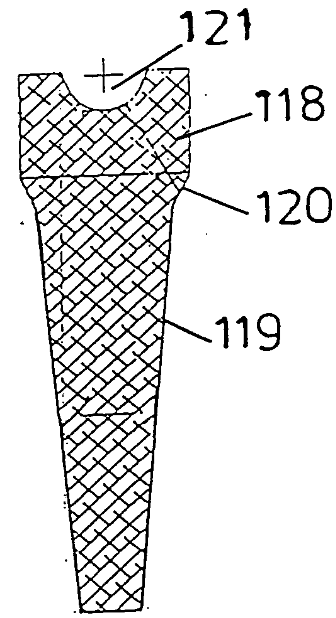

FIG. 2

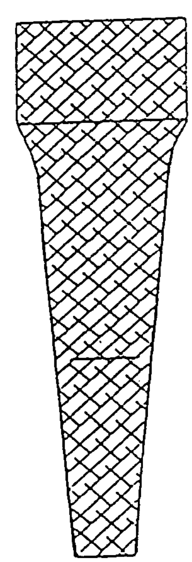
FIG. 3

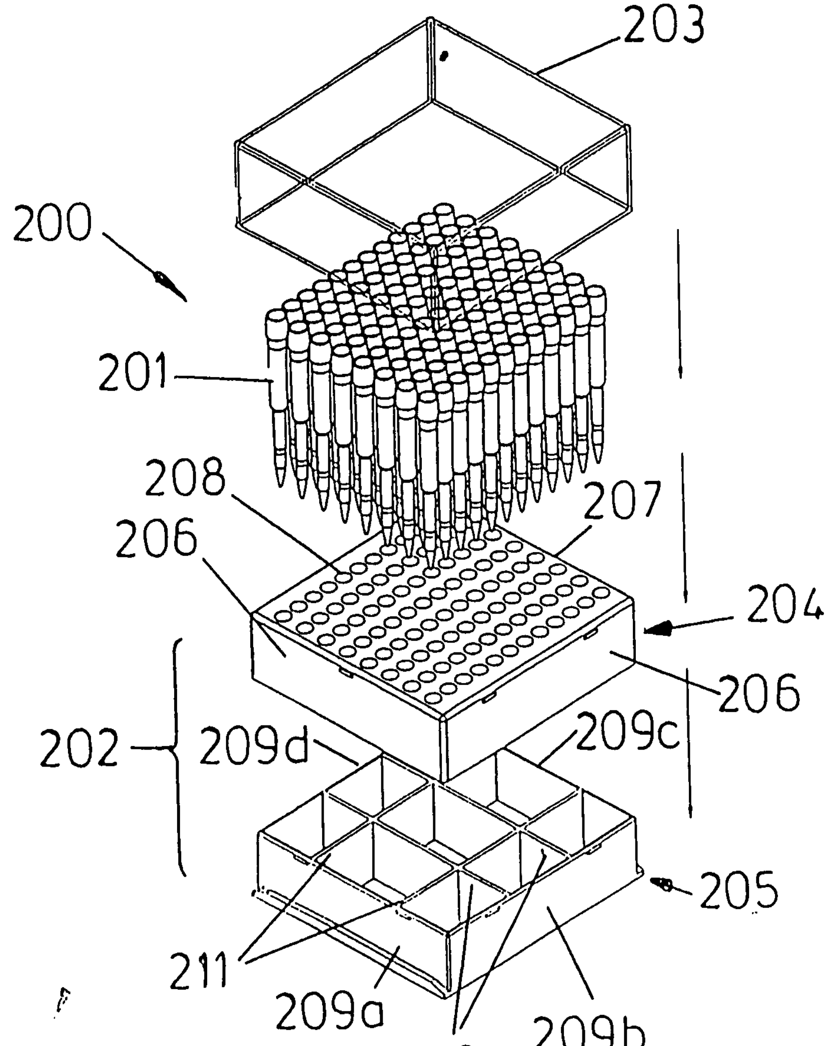
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FIG.4a

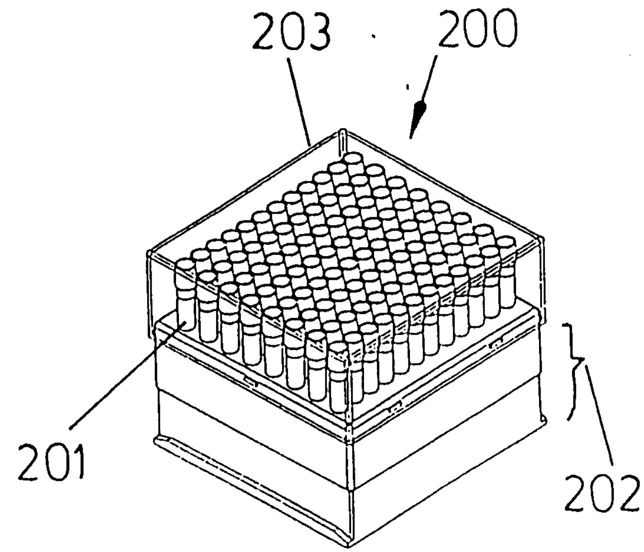
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FIG.4b

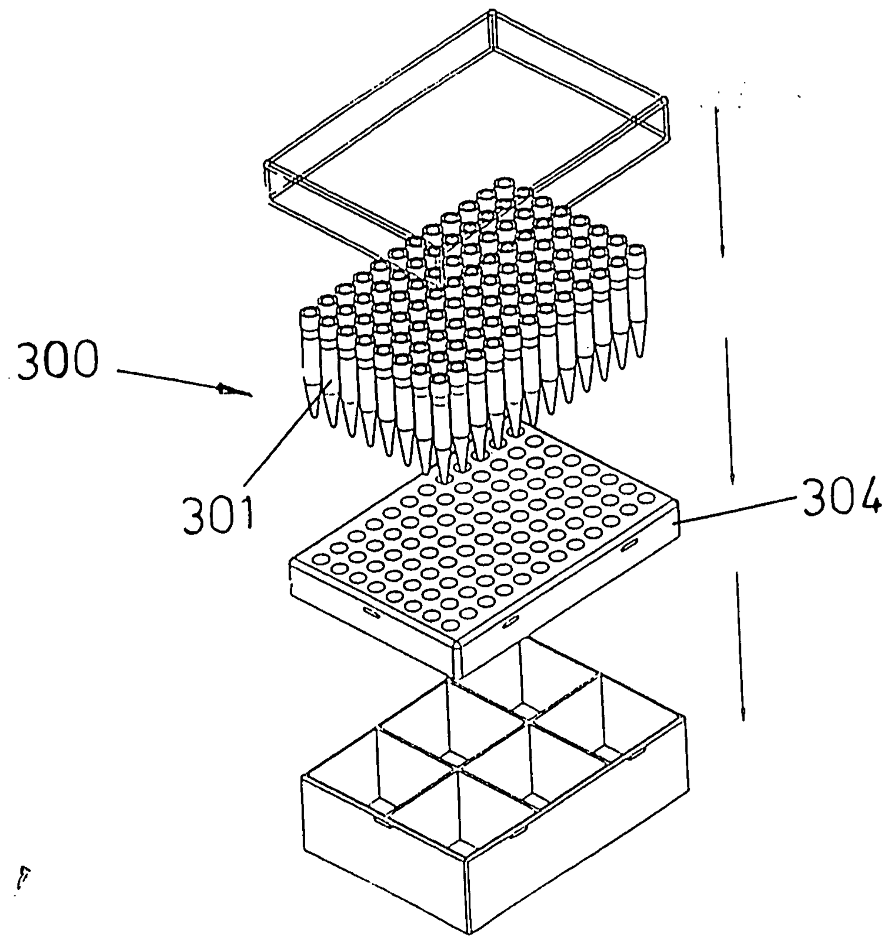

FIG. 5a

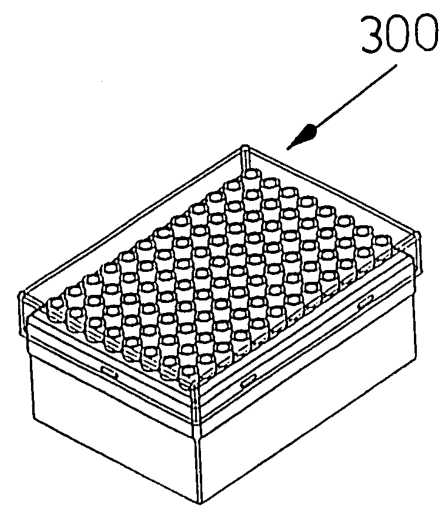

FIG. 5b

APPENDIX

ISOLATION OF BIOMOLECULES

The present invention relates to a purification method and apparatus for use in obtaining samples of biomolecules. The invention relates more particularly, but not exclusively, to such method and apparatus which may be used for obtaining samples of nucleic acids or proteins.

Numerous methods are known for obtaining biomolecules, for example nucleic acids and proteins, from biological material such as viruses, bacterial and eukaryotic cells, cell aggregates and tissue or body fluids. Typically the biomolecule to be obtained is a soluble molecule and is "released" from the biological material by a lysis procedure (e.g. alkaline lysis) resulting in a suspension comprised of a solution of the target biomolecule also containing soluble proteins, carbohydrates, fats, amino acids and other metabolites from the disrupted cells.

In many methods known in the art for effecting the purification of a desired biomolecule, those molecules which would otherwise contaminate the desired product are insoluble, or are rendered so by a chemical process. The insoluble material is then removed by methods known in the art (e.g. centrifugation and aspiration of the supernatant) to achieve a degree of purification of the soluble material. These insoluble materials may include, for example, whole cells, or fragments thereof, flocculated proteins and unwanted nucleic acid material (e.g. chromosomal DNA contamination of a plasmid DNA preparation).

After separation of the insoluble material, from the solution, the latter is applied to a solid phase binding matrix under conditions (e.g. in the presence of a chaotropic salt) such that the matrix binds the biomolecule of interest. Subsequently the solution is removed from the matrix (leaving the biomolecule bound thereto), the matrix washed to remove non-bound material, and the biomolecule eluted.

The separation of the insoluble material from the solution is generally effected by centrifugation of the suspension followed by careful pipetting. However the use of centrifugation is a disadvantage in that it does not easily allow the procedure for obtaining the biomolecule to be fully automated. Furthermore, the separation of soluble from insoluble material by pipette is awkward to perform and must be carried out accurately to prevent unwanted insoluble material from being added to the solid phase matrix and causing contamination.

One purification method which seeks to overcome some of the problems associated with conventional techniques is disclosed in WO-A-95/02049. The apparatus disclosed involves a pneumatic delivery system which is used to add, mix and remove reagents from a flow-through vessel in order to separate a target biomolecule from cells. The vessel has two chambers between which is a porous membrane or filter. The membrane functions to retain cells and cellular debris and insoluble material in the upper chamber whilst soluble material is filtered through and further purified by binding to a solid phase matrix present in the lower chamber.

There are however disadvantages associated with the technique disclosed in WO-A-95/02049. In particular, if it is desired to treat the solid phase matrix (with biomolecule bound thereto) with additional solutions (e.g. wash or elution solutions) it is necessary to pass those solutions into the lower chamber either via the upper chamber and filter (which retains insoluble material) or provide additional inlets in the lower chamber. In the former case, there is the risk of contamination. In the latter case, the provision of additional ports in the lower chambers makes the apparatus difficult to manufacture.

It is an object of the present invention to obviate or mitigate the aforementioned disadvantages.

According to a first aspect of the present invention there is provided a method of obtaining a sample containing the biomolecule from a suspension comprising a solution of the biomolecule and insoluble material, the method comprising the steps of:

(a) providing a biomolecule purification assembly comprised of a vessel having a liquid inlet and of a filter unit removably located on the liquid inlet;

(b) effecting a filtration of the suspension through the filter unit so as to cause the solution to enter the vessel through the liquid inlet;

(c) removing the filter unit from the liquid inlet;

(d) immobilising the biomolecule on a solid phase support; and

(e) subjecting the molecule to at least one of the steps of washing on the support and elution from the support to obtain a purified sample of the biomolecule.

According to a second aspect of the present invention there is provided apparatus for obtaining a sample of a biomolecule from a suspension comprising a solution of the biomolecule and insoluble material, the apparatus comprising

(i) a biomolecule purification assembly comprised of a vessel having a liquid inlet and of a filter unit removably located on the liquid inlet of the vessel;

(ii) means for causing the solution present in the suspension to pass through the filter unit and the liquid inlet into the vessel; and

(iii) means for removing the filter unit from the vessel.

In accordance with the invention therefore separation of insoluble material from the suspension is effected through a filter unit which is removably located in the liquid inlet of a vessel and a filtration is effected causing the solution to enter the vessel. Any insoluble impurities in the suspension are retained on the filter unit. Subsequently, the filter is removed from the inlet and is preferably discarded rather than being reused. Thus each filter need only be used once avoiding problems of contamination.

The method of the invention negates the requirement for the suspension to be centrifuged to separate soluble and insoluble materials in the suspension. The method further negates the requirement for accurate separation of the soluble and insoluble phases by use of a pipette. Furthermore, the method of the invention is relatively simple to perform and is eminently suited to automation as will be appreciated from the description given below.

It is preferred that, in step (e) of the method, the step of washing or elution is effected on solid phase support contained within the vessel. Preferably both of the steps of washing and elution are effected, and the biomolecule of interest is eluted through said inlet of the vessel.

It is particularly preferred that the solid phase support material is a particulate or bead-like material which is introduced into the vessel (preferably through said inlet) after the step of removing the filter unit therefrom.

The vessel of the biomolecule purification assembly is preferably a flow through vessel, most preferably an open-ended column, e.g. having a volume sufficient to hold 0.25 to 1.5ml of sample, disposed vertically so that its lower end provides the aforementioned liquid inlet and the upper end may be used for the introduction of additional reagents into the column as required.

The vessel may comprise upper and lower bore sections whereof the diameter of the upper section is greater than that of the lower section, the two sections being connected by a intermediate, tapering bore section, the purpose of the reduced section lower bore will be described below.

The filter unit may be of any material capable of tolerating the reagents used and will comprise a filter element having a pore size which is capable of preventing passage of the insoluble material of the composition therethrough but which is not so small that the flow rate through the filter becomes unacceptably low. Typically the pore size of the element will be in the range 0.2 to 50 microns.

The filter unit may, for example, comprise a sleeve or the like for location over the liquid inlet of the vessel and a filter located in the sleeve. The filter unit may for example be a push fit or a loose snap fit over the liquid inlet.

Preferably the filter incorporates a depression locating in close proximity to the liquid inlet 8 of the vessel. This allows filtrate to come into close proximity with the inlet and enhance the rate of filtrate uptake into the vessel.

The filtration step of the method of the invention may be effected in a number of ways. Thus for example, a reduced pressure may be applied to the interior of the vessel causing solution from the suspension to be drawn through the filter unit into the vessel. For example, when the biomolecule of interest is a nucleic acid molecule a chaotropic salt will generally be required to allow the nucleic acid molecule to bind to the matrix. The chaotropic salt may be provided with the suspension to be applied to the matrix or may be pre-equilibrated with the matrix prior to contact of the filtered solution with the matrix. Subsequently, excess solution may be discharged from the vessel.

As an alternative to the use of reduced pressure, the suspension may be provided in an open-topped container within which the filter unit is a close sliding fit such that by moving the filter unit within the container towards the base thereof solution is caused to be forced through the filter unit into the vessel for binding of the biomolecule as described above.

Subsequent to the filtration operation, the filter unit is removed from the vessel.

It is particularly preferred in accordance with the invention that the suspension to be filtered is contained in a well and that once the filtration operation is complete the filter unit is automatically discarded into that well. This may be achieved in a number of ways. Thus, for example, the biomolecule purification assembly may be lowered towards the well to effect filtration and move upwardly after filtration is completed and the apparatus may incorporate a stripping arrangement which acts on the filter unit as the biomolecule purification assembly is moved upwardly causing the filter unit to be discharged into the well. Alternatively, the filter unit and microtitre well have inter-engagable formations whereby as the biomolecule purification assembly is lowered towards said well the formations come into an engaging relationship requiring a greater force to release the engagement than is required for removing the filter unit from the vessel. Thus, as the vessel is moved upwardly away from the well, the engagement is maintained and the filter unit is removed from the vessel and retained in the well.

Subsequent to the removal of the filter unit, the biomolecule is immobilised in a solid phase support (i.e. step (d) of the method), preferably in the form of particles or beads having a size of 0.1 to 250 microns Preferably the particles do not fill the entire space between the retaining means so that the particles may be "fluidised" within the vessel.

The immobilisation of the biomolecule onto the solid phase support is preferably effected in the presence of a chaotropic salt or other agent(s) capable of effecting absorption of the biomolcule onto the support. By chaotropic salt it is meant any substance capable of altering the secondary, tertiary and/or quaternary structure of a protein or nucleic acid molecule, but leaving at least the primary structure intact. Examples of chaotropic salts which may be utilised to allow binding of nucleic acid of proteins to the solid phase binding matrix are guanadinium salt, sodium iodide, potassium iodide, sodium (iso)thiocyanate, urea or combinations thereof. Preferred chaotropic salts for use in the present invention include guanidinium hydrochloride and guanidinium (iso)thiocyanate. For the purposes of effecting step (d) of the method (i.e. immobilising the biomolecule on the solid phase support), the filtrate in the vessel may contain the chaotropic salt or other immobilising agent(s) and the filtrate is discharged onto the support material prior to the resultant mixture being taken back into the vessel. Alternatively, the filtrate may be discharged into a mixture of the support and the chaotropic salt or other agent(s) and the resultant mixture is then taken back into the vessel.

Subsequently, the mixture of the filtrate, chaotropic salt (or other immobilising agent(s)) and the beads may be drawn back up into the vessel and, if desired, may be subject to at least one cycle of discharge from, and uptake back into, the vessel to improve mixing.

It is preferred that the support comprises magnetic beads.

The magnetic beads within the vessel may be manipulated by a magnet positioned externally of the vessel.

Step (e) of the method may be effected in a number of ways.

Thus, for example, a magnet may be used to "hold" the magnetic beads (with immobilised biomolecule) at the side of the vessel. In the case of the preferred biomolecule purification assembly in which the vessel has a reduced diameter bore section, it is preferred that the beads are "held" at this position in the vessel.

Subsequently, the solution may be discharged from the vessel, wash buffer introduced into the vessel, and the magnet manipulated to re-suspend the beads in the buffer. The magnet may once again be used to hold the beads in place and the wash buffer discharged from the vessel.

The washing operation may be repeated at least once. For preference the final wash step employs 70% ethanol (in water).

After removal of the final wash solution from the vessel, (and with the beads being held therein by means of the magnet), air may be passed over the beads to effect drying. Alternatively or additionally heat may be applied to the vessel, e.g. by locating the latter in a heating block.

Subsequently, the biomolecule may be eluted from the vessel. This may be achieved by introducing an elution buffer into the vessel, admixing the particles with the buffer, heating the admixture, immobilising the particles by means of the magnet, and discharging the solution (containing dissolved biomolecule) from the vessel for collection and subsequent processing

A particularly preferred embodiment of apparatus in accordance with the invention is capable of handling an array of biomolecule purification assemblies and therefore each of the individual steps (described above) of filtration, removal of the filter unit, "pick up" of magnetic particles, washing and elution is effected simultaneously on all members of the array.

In such an apparatus, it is preferred that the vessels (of the biomolecule purification assemblies) are vertically disposed, open-ended columns. The upper ends of such columns may be associated with pumps for applying reduced pressure to the interior of the column for drawing liquid into the columns (e.g. for the purposes of the filtration and washing and elutiön operations) and for blowing or drawing drying air through the columns. Furthermore, the upper ends of the vessels may be associated, via appropriate valving arrangements, with appropriate reagent reservoirs permitting reagents to be passed downwardly into the columns if required.

A particularly preferred embodiment of apparatus in accordance with the invention for use in conjunction biomolecule purification assembly's as defined in the previous paragraph comprises separate filtration, bead "pick up", washing and elution stations Thus, at the filtration station, there may be a first set of wells (e.g. a BioBlock) each containing an aliquot of the suspension and each member of the array effects filtration of an aliquot from the corresponding well at the station. The apparatus may be such that the filter unit of the biomolecule purification assembly is discharged into the respective well at the filtration station. At the bead "pick up" station, there may be a second set of microtitre wells each containing magnetic beads in a chaotropic solution. At the wash station, wash solution may be passed to the vessel (e.g. by pumping) and discharged through the liquid inlet into a waste receptacle which may be connected to a drain. Furthermore, the elution station may have a second set of microtitre wells containing elution buffer into which the filtrate/magnetic beads are discharged prior to being taken back up into the vessel. The final step of elution is then effected by holding the magnetic beads in position and discharging the liquid containing the desorbed biomolecule from the vessel. Obviously, the apparatus will include a magnet or magnets as necessary for manipulating the magnetic beads.

In a particularly preferred embodiment of the apparatus as described in the previous paragraph, the apparatus incorporates a head arrangement capable of selectively "picking-up" and releasing 12 of the biomolecule purification assemblies.

Furthermore, the wells at the various stations may each be provided by a 12 by 8 array of microtitre wells so that in any one cycle of the apparatus a total of 12 samples may be processed. By operating the apparatus through 8 cycles then a total of 96 samples may be processed before the microtitre wells need to be replaced.

It is also preferred that the apparatus incorporates an upstream station at which the head arrangement is capable of "picking-up" the biomolecule purification assemblies to be used in any one cycle of the apparatus and a final discharge station at which the vessels of the assemblies are discarded. Thus, if the head arrangement is capable of holding 12 biomolecule purification assemblies then 12 such assemblies are "picked-up" at the beginning of each cycle of the machine.

Such an apparatus will further comprise mechanisms for moving those parts of the apparatus as are required to complete the method of the invention in the directions (X,Y and/or Z) so to do. The apparatus as described above may readily be automated and is capable of operating under the control of a programmed microprocessor.

The method and apparatus of the invention are particularly suitable for obtaining a sample of a biomolecule from a suspension obtained by a lysis procedure (e.g. a standard alkaline lysis procedure as well known to those skilled in the art) effected on a biological material.

The target biomolecule may, for example, be a nucleic acid (DNA or RNA) and may for example be a semi-purified or non purified, native or synthesised nucleic acid. The target soluble biomolecule may be any DNA or RNA sequence from a viral, bacterial, animal or plant source. Apart from the utility in purifying DNA and RNA samples and especially for purifying plasmid DNA and other recombinant DNA constructs, such as phagemids, free from chromosomal DNA, the method and

apparatus according to the present invention are also suitable for isolating recombinant proteins and antibodies, especially from cellular samples

The biological material on which the lysis is effected may for example comprise cells. For the purposes of the present specification, the term "cell" is intended to encompass bacterial cells, cells (e.g. blood cells) from higher organisms, virus particles and other cell types or organelles which contain the target biomolecule and which may be released in a soluble form by a lysis procedure.

In the case of bacteria, the nucleic acid to be isolated may be from the bacterial genome or from an extragenomic element such as a plasmid. Phage infected bacteria may also be used for the preparation of phage DNA, such as M13 DNA.

According to a preferred embodiment of the present invention the composition from which the target biomolecule is to be isolated is a lysed and neutralised bacterial cell composition containing soluble plasmid DNA and insoluble precipitated genomic DNA, flocculated protein and other cellular debris.

In order to isolate plasmid DNA that has been propagated within bacterial hosts, the following steps may be followed:

i) growth of bacterial host in an enriched medium;

ii) centrifugation of bacteria to form a pellet after which the supernatant is discarded;

iii) resuspension of the bacterial pellet in a buffered solution;

iv) addition of lysis reagent which releases the cellular contents; and

v) addition of neutralisation solution which causes the formation of suspension comprising a solution containing dissolved plasmid.

In a particularly advantageous implementation of the invention, the resuspension obtained from step (iii) may be provided. e.g. in a microtitre well, to the

apparatus which is adapted to be such as to add lysis solution to the resuspension. In the preferred embodiment of the invention, in which the vessels (of the biomolecule purification assemblies) are vertically disposed, open-ended columns, the apparatus comprises a 'column-head' on which the biomolecule purification assemblies are to be mounted. However, prior to mounting of the assemblies on their respective heads, lysis solution may be injected ("fired") from the head into the resuspension in the micro-titre well. The neutralising solution may be added in the same way. This will also provide for good mixing of the lysis solution and neutralising solution with the resuspension. Subsequently, the biomolecule purification assemblies are mounted on their respective heads for effecting separation, washing and elution procedures as described.

In an alternative implementation, the lysis solution (drawn from a reservoir thereof) may be ejected downwardly through the column into the resuspension prior to mounting of the filter unit on the column.

In both cases the inventors have found that introduction of the lysis solution and neutralising solution in this way into the resuspension avoids the need for any further mixing so lysis and neutralisation can be effected. Furthermore, the introduction of the lysis and neutralising solution is effected without the dispensing apparatus coming into contact with the sample or its containing vessel.

In a particularly preferred embodiment of the invention firing of multiple aliquots of lysis solution or neutralising solution into the resuspension well provides better mixing (or at least more efficient extraction of product) than a single addition of reagent.

The invention will be further described, by way of example only, with reference to the accompanying drawings, in which:

Fig. 1 schematically illustrates a first embodiment of the invention;

Fig 2 schematically illustrates the column of the assembly shown in Fig 1;

Fig 3 illustrates the sleeve of the filter unit in Fig 1; and

Fig. 4 schematically illustrates to an enlarged scale the filter of the assembly shown in Fig 1.

Referring firstly to Fig. 1, there is illustrated a biomolecule purification assembly 1 for use in obtaining a purified sample of a biomolecule of interest from a suspension (e.g. as obtained by an alkaline lysis procedure) comprising a solution of the biomolecule containing insoluble biological debris. The illustrated assembly 1 comprises a vertically disposed, open-ended column 2 and a filter unit 3.

Referring to Fig 2, column 2 is referenced for convenience as being comprised of body sections 4, 5, 6 and 7. Body section 4 defines an upper cylindrical bore 4a which at its lower end is connected to a downwardly tapering section 5a leading into a lower bore 6a which is of reduced diameter as compared to bore 4a. At its lower end bore 6a leads into a tapering section 7a defined within the lower section 7 of the column 2.

The lower end of tapering section 7a defines a liquid inlet 8 for the column.

At the upper end of column 2 there is provided a formation 9 by means of which the column may be mounted on the head of a sample processing apparatus of the type described more fully above.

Referring back to Fig 1, the filter unit 3, is a two component part and comprises a sleeve 10 (see Fig 3) having an internal bore 11 within which is housed a filter 12(see Fig 4) having depression 13 in its upper surface. Filter 11 is such that it is permeable to liquids but is capable of filtering the insoluble debris in the suspension from which the biomolecule is to be obtained.

Sleeve 10 is removably mounted on column 2 and more particularly is located over section 7 and the lower end of section 6 thereof. With the filter unit 3 located in position, the lowermost portion of section 7 of column 2 locates in the depression 13 in the upper surface of the filter 12 allowing the filtrate to come into proximity with the liquid inlet 8. As a result, the rate of filtration is enhanced.

CLAIMS

1. A method of obtaining a sample of a biomolecule from a suspension comprising a solution containing the biomolecule and insoluble material, the method comprising the steps of:

(a) providing a biomolecule purification assembly comprised of a vessel having a liquid inlet and of a filter unit removably located on the liquid inlet;

(b) effecting a filtration of the suspension through the filter unit so as to cause the solution to enter the vessel through the liquid inlet;

(c) removing the filter unit from the liquid inlet;

(d) immobilising the biomolecule on a solid phase support; and

(e) subjecting the biomolecule to at least one of the steps of washing on the support and elution from the support to obtain a purified sample of the biomolecule.

2. A method as claimed in claim 1 wherein the step (e) comprises washing the solid phase support and said support is contained within the vessel during this step.

3. A method as claimed in claim 2 wherein step (e) comprises eluting the biomolecule from solid phase support contained within the vessel, the elution being effected through said inlet of the vessel.

4. A method as claimed in any one of claims 1 to 3 wherein the step (b) the suspension to be filtered is contained in a well and in step (c) the filter unit is discharged into that well.

5. A method as claimed in any one of claim 1 to 4 wherein the biomolecule is absorbed onto the solid phase support in the presence of the chaotropic salt or other agent(s) for effecting said adsorption.

6. A method as claimed in claim 5 wherein, for the purposes of step (d) the filtrate in the vessel contains the chaotropic salt or other agent and the filtrate is discharged onto the support and the resultant mixture is taken back into the vessel.

7. A method as claimed in claim 5 wherein, for the purposes of step (d), the filtrate is discharged into a mixture of the support and the chaotropic salt or other agent(s) and the resultant mixture is taken back into the vessel.

8. A method as claimed in any one of claims 1 to 8 wherein the solid phase support comprises magnetic beads.

9. A method as claimed in any one of claims 1 to 8 wherein the vessel of the biomolecule purification assembly is an open-ended, vertically disposed column.

10. A method as claimed in claim 9 wherein the column has an upper bore section and a lower bore section of reduced diameter as compared to the upper section.

11. Apparatus for obtaining a sample of a biomolecule from a suspension comprising a solution of the biomolecule and insoluble material the apparatus comprising

(i) a filtration station at which is provided a biomolecule purification assembly comprised of a vessel having a liquid inlet and a removable filter unit located at the inlet of the vessel, said filtration station being provided with means for causing the

solution present in the suspension to pass through the filter unit and the liquid inlet into the vessel;

(ii) means for removing the filter unit from the vessel; and

(iii) at least one of a washing station and an elution station.

12. Apparatus as claimed in claim 11 comprising an elution station and a washing station.

13. Apparatus as claimed in claim 11 or 12 further comprising a solid phase support supply station preferably provided between (ii) and (iii).

14. An apparatus as claimed in any one of claims 11 to 13 wherein the vessel is an open-ended flow-through column.

15. An apparatus as claimed in claim 14 wherein the column has an upper bore section and a lower bore section of reduced diameter compared to the upper section.

16. An apparatus as claimed in claim 14 or 15 wherein the filter unit comprises a sleeve which incorporates the filter and which is located over the lower end of the column.

17. An apparatus as claimed in claim 16 wherein the upper surface of the filter has a depression and the lower end of the column locates in the depression.

18. An apparatus as claimed in any one of claims 11to 17 in which the pore size of the filter is in the range 0.2 to 50 microns.

19. An apparatus according to any one of claims 11to 18 in which the suspension to be filtered is contained in a well and once the filtration operation is complete the filter unit is automatically discarded into that well.

20. An apparatus according to claim 19 further comprising a stripping arrangement which acts on the filter unit when the assembly is moved upwards causing the filter unit to be discharged into the well.

21. An apparatus according to any one of claims 11to 20 which is capable of handling an array of biomolecule purification assemblies.

22. An apparatus according to claim 21 wherein each individual step of filtration, removal of the filtration unit washing and elution is adapted to be effected simultaneously on all members of the array.

23. A biomolecule purification assembly comprising a vessel having a liquid inlet and a filter unit removably located on the liquid inlet wherein the vessel is in the form of a column having a first bore section and a second bore section of reduced diameter as compared to the first section, and the filter unit is comprised of a sleeve which houses the filter and which is removably located on the end of the vessel remote from the first bore section.

24. A biomolecule purification assembly as claimed in claim 22 wherein the surface of the filter adjacent to the inlet of the vessel has a depression and the end of the vessel locates in the depression.

25. A method as claimed in claim 1 wherein the suspension is obtained by the steps of:

(i) providing a re-suspension of a bacterial pellet in solution;

(ii) adding lysis solution to the re-suspension to effect release of cellular contents; and

(iii) adding neutralising solution to the lysed re-suspension to effect the formation of a suspension comprising a solution of a biomolecule and insoluble material for use in the method of claim 1;

in which the lysis solution and/or neutralising solution are each added in multiple aliquots which are ejected downwardly into the re-suspension to provide the suspension in the absence of additional mechanical agitation or stirring without the dispensing apparatus coming into contact with the sample or the containing vessel.

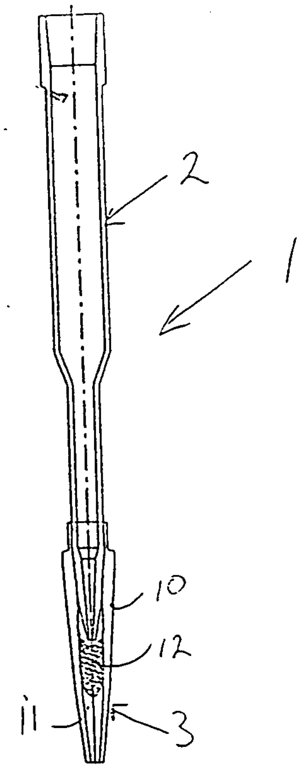

FIG 1

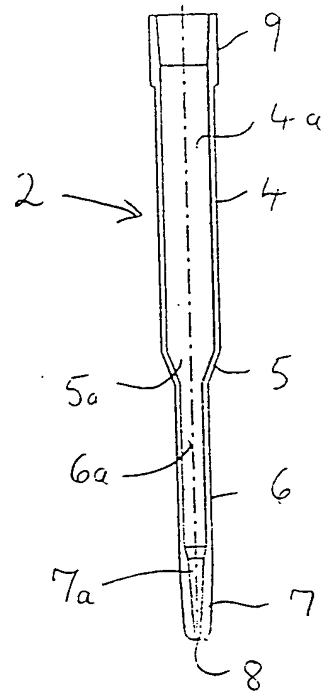

FIG 2

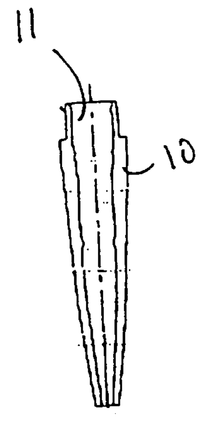

FIG 3

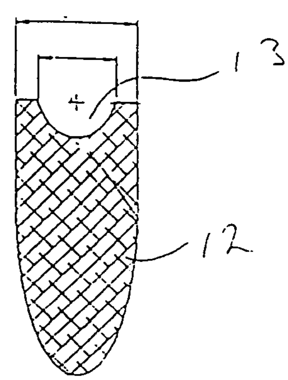

FIG 4

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